Genetic Identity and Diversity of Perennial Pepperweed (*Lepidium latifolium*) in Its Native and Invaded Ranges

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Perennial pepperweed is an invasive plant species in North America, native to temperate Eurasia and northern Africa. Effective biological control depends upon correct taxonomic identification. Therefore, we investigated morphological and genetic data (cpDNA sequences and amplified fragment length polymorphisms [AFLP]) in its native range, where the species is at times treated as multiple taxa (*L. latifolium, L. affine* and *L. obtusum*). We also analyzed genetic data to determine the number and distribution of haplotypes and genotypes in the invaded range. Using Bayesian analysis, we found three clusters of AFLP genotypes in the native range, but little correlation between these clusters and morphological characters used to distinguish taxa. Also, we found combinations of morphological character states within many native range plants that are incompatible with current species descriptions, offering no support for splitting *L. latifolium* sensu lato into three species. In North America 97% of the genetic variation was among populations and there were only eight AFLP genotypes in 288 plants, suggesting few introductions or a severe bottleneck, and little or no creation of new genotypes since introduction. We found plants in the native range that are genetically similar (88 to 99%) to six of the eight invasive AFLP genotypes, suggesting that Kazakhstan and China are origins for much of the North American invasion.

Nomenclature: Perennial pepperweed, *Lepidium latifolium* L. LEPLA, *Lepidium affine* Ledeb., *Lepidium obtusum* Basiner.

Key words: AFLP, DNA sequence, invasive, morphology, weed.

Classical biological control of weeds aims to find and import host specific co-evolved arthropods or fungal pathogens from a plant's native range that can help manage the plant where it is invasive. Clear taxonomic identification of the invasive plant in its native and introduced ranges is a prerequisite for the success of any biological control project (Gaskin et al. 2011). However, this can be difficult if different taxonomic systems are used in the native and introduced ranges, or if hybridization or phenotypic plasticity complicates identification (De Queiroz 1999, Helbig et al. 2002, Hrusa and Gaskin 2008, Rieseberg et al. 2006). Misidentification of an invasive plant in either its introduced or native ranges can lead to searches for biological control agents on the wrong taxa or outside of the invasive's native range (Estoup and Guillemaud 2010; Goolsby and Moran 2009), lowered agent efficacy (Burdon et al. 1981), host plant resistance to agent attack (Campanella et al. 2009; Lym and Carlson 2002), or selection of an agent with a broad fundamental host range (Schaffner 2001; Van Klinken and Edwards 2002), any of which could lead to the risk of nontarget plants being attacked. Proper taxonomic identification is also critical when there are taxa morphologically similar to the target plant in the invaded or native range, or to distinguish hybrids (Gaskin and Kazmer 2009; Moody and Les 2007; Saltonstall 2002). Even if the identification is correctly determined, invasions can contain diverse assemblages of

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Management Implications

Clear taxonomic identification of an invasive plant in its native and introduced ranges is a prerequisite for the success of any biological control project. Also, invasions can contain diverse assemblages of genotypes, which can affect herbivory. To verify the identity of perennial pepperweed populations from Eurasia that are being explored for biological control agent candidates we collected and analyzed morphological and genotypic data from populations in Eurasia and North America. In addition, we analyzed genotypic data to determine the diversity and distribution of perennial pepperweed genotypes in North America. Our results indicate that perennial pepperweed most probably consists of one taxon; not two or three as suggested in some recent floristic studies. We found that origins of common invasive genotypes are in Kazakhstan and China, suggesting that these regions should preferentially be searched in future foreign exploration for additional biological control agents. We found only eight invasive genotypes in North America, suggesting few introductions or a severe bottleneck, and little or no creation of new genotypes since introduction. The low diversity allowed us to provide seed representing all cpDNA sequence haplotypes for host-specificity studies, which should reduce the risk of any resistance to biological control agents being found after release.

genotypes, which can affect control (Blair et al. 2008; Fritz et al. 1994). Population biology tools, often molecular-based, provide insight into variation within invasive species in their introduced range that may be driven by founding events, bottlenecks, postintroduction hybridization, or distinct origins of genotypes and lineages (Ellstrand and Schierenbeck 2000; Goolsby et al. 2006; Lee 2002; Petit 2004; Sakai et al. 2001; Ward et al. 2008). Using these tools to characterize the amount and distribution of genetic diversity among and within invasive populations can enhance the efficacy of biological control programs (Gaskin et al. 2011; Müller-Schärer et al. 2004) by ensuring that appropriate genotypes are used in host specificity testing, so that any resistance or tolerance by the invasive plants to candidate biological control agents will be noted prior to agent release. Knowledge of the geographical distribution of genotypes within an invasive species also determines where to apply agents if some are only effective on a subset of the invasive plant genotypes (Burdon et al. 1984; Gaskin et al. 2011; Ward et al. 2008).

Perennial pepperweed (*Lepidium latifolium* L.), originating from temperate Eurasia and northern Africa, is an invasive species found in all western states and in parts of the northeastern United States, as well as Quebec and western Canada, with smaller invaded areas in Mexico and Australia (Rios and Garcia 1998; USDA 2005; Zouhar 2004). The species is thought to have been introduced to the western United States as a contaminant of sugarbeet seed from Europe (Robbins et al. 1952). Its distribution and dominance in the United States has greatly increased since the 1980s (Young et al. 1998). Patches of this species often spread vegetatively to dominate local areas (Renz et al. 2012). The subsequent decrease in native plant diversity after perennial pepperweed invasion lowers habitat quality and alters ecosystem functions (Zouhar 2004). Dense stands of perennial pepperweed interfere with regeneration of native willows and cottonwoods along rivers (Young et al. 1995) and displace threatened, endangered, and rare species such as the salt marsh harvest mouse (*Reithrodontomys raviventris* Dixon) (Trumbo 1994) and Suisun Marsh aster (*Aster lentus* Greene) (Skinner and Pavlik 1994). Perennial pepperweed is difficult to control because of its large, stout rhizome system, and neither mechanical nor chemical control techniques appear to provide permanent solutions (Young et al. 1998). In 2006, a biological control project for perennial pepperweed was started and surveys for specific natural enemies in the native range of the plant were conducted.

Perennial pepperweed is found throughout most of Europe, except for the northernmost countries (Henman 2003; Tutin et al. 1976), and southeast to central and western Asia (Cheo et al. 2001; Hedge 1965) as well as northern Africa. Presence in western Europe and the Mediterranean is most likely because of its historic cultivation as a vegetable and spice (Francis and Warwick 2007). A center of morphological diversity apparently lies in Tajikistan, Kazakhstan and western Mongolia, and taxonomic studies distinguish three species in this area: *Lepidium latifolium*, *L. affine* Ledeb. and *L. obtusum* Basiner (Bush and Komarov 1970; Czerepanov 1995; see Table 1) based on cauline leaf tip angle, pedicel pubescence, sepal persistence, shape of fruit base, ultimate fruiting branch structure, cauline leaf attachment and fruit surface pubescence. The Flora of China (Cheo et al. 2001) recognizes *L. latifolium* and *L. obtusum* but suggests that morphologies attributed to *L. affine* and *L. latifolium* are found mixed in populations, and thus does not recognize *L. affine*. Another taxon, *L. sibiricum* Schweigg., is considered to be a form of *L. latifolium* and is not typically treated as an accepted name (Anonymous 2012; Jafri 1973). Although five sub-specific taxa of *L. latifolium* have been described (Anonymous 2012), most recent taxonomic treatments do not recognize the splitting of *L. latifolium* into subspecies or varieties (see p. 640 of Francis and Warwick 2007). In the United States, perennial pepperweed is only known under the name *L. latifolium.* The high morphological variability and unclear taxonomic identity of perennial pepperweed has complicated recent surveys for potential biological control agents in the native range where all three putative taxa are sympatric, leaving researchers unsure if they were searching for natural enemies on the correct plants, especially when these were not yet bolting or reproducing (H. Hinz pers. communication). Thus, our first goal was to verify the identity of perennial pepperweed populations from the native range that are being explored

Table 1. Key to *Lepidium latifolium* and putative conspecific taxa, derived from the treatment and discussion found in Cheo et al. (2001; Flora of China).

1. Cauline leaf tip obtuse or acute; pedicel pubescent; sepals persistent in fruiting stage; fruit base cordate; ultimate fruiting branches capitate, 2n = 16	*Lepidium obtusum* Basiner
1. Cauline leaf tip acute; pedicel glabrous; sepals deciduous in fruiting stage; fruit base blunt, round; ultimate fruiting branches subcapitate, 2n = 24	2
2. Upper cauline leaves subsessile; fruit surface pubescent	*Lepidium latifolium* L.
2. Upper cauline leaves sessile; fruit surface glabrous	*Lepidium affine* Ledebour

for biological control agent candidates, using morphological and genotypic data from populations in Eurasia and North America. To further enhance the efficacy of biological control, our second goal was to analyze haplotypic and genotypic data and determine the diversity and distribution of perennial pepperweed in North America.

Materials and Methods

Plant Material. We collected or received leaf material from 1 to 17 perennial pepperweed plants each from 44 populations from its invasive range in North America and from 54 populations from its native range in Eurasia (Figures 1a,b). Leaves were stored in silica gel until processing and a herbarium specimen was collected for most populations (Appendix 1). In North America, sampling was concentrated in the western and midwestern United States, where perennial pepperweed is most invasive, but five populations from the eastern United States, where it is not as common, were also included. In the native range, we focused on a region where all three putative taxa (*L. latifolium*, *L. affine* and *L. obtusum*) are sympatric, i.e. western China and eastern Kazakhstan, and also included plants from regions where only *L. latifolium*, and not *L. affine* or *L. obtusum*, should be present (viz. Armenia, Turkey, southwestern Russia, Iran, and Switzerland). We sequenced the chloroplast *trnS* (GCU)- *trnG* (UCC) spacer of 230 plants; 91 plants from 41 populations in North America (invaded range), and 139 plants from 38 populations in Eurasia (native range). We typically sequenced two plants from most invasive and native populations, especially native populations with *L. affine* or *L. obtusum* morphological character states, as these were rare compared to *L. latifolium*. To determine genetic variability within populations we used AFLPs, as these evolve more quickly than the sequence marker, for 431 plants; 288 invasive plants from 30 populations in North America and 143 plants from 14 populations in Eurasia. To record morphological characters that have been used to separate the different taxa in the native range, herbarium specimens were collected from 24 locations in North America, 13 from Kazakhstan and 17 from China. Unfortunately, mature plants for morphological comparison were not available for all populations. Collection information, including which plants were subject to sequencing, AFLP and morphological analysis, is listed in Appendix 1.

Sequencing. DNA extraction and sequencing of the *trnS-G* spacer (Hamilton 1999) was performed as in Gaskin et al. (2005). Sequencing was performed on an Applied Biosystems 3130 Genetic Analyzer. Each distinct haplotype sequence is listed in Appendix 2 (FASTA format).

AFLPs. The AFLP method followed Vos et al. (1995) with modifications as in Gaskin et al. (2012). We used individually ordered reagents instead of a kit. Each selective PCR product was combined with 0.25 µl of 600 base pair (bp) size standard and 9.25 µl of de-ionized formamide and loaded into an Applied Biosystems 3130 Genetic Analyzer. All selective primer combinations of *Mse*I + CAA, CAC, CAT, CTA, or CTA and *Eco*RI + AAG, ACC, or ACT were prescreened for 8 samples, and the two most polymorphic primer pairs were chosen (*Mse*I + CAT/*Eco*RI + AAG and *Mse*I + CTA/*Eco*RI + AAG).

Data Analysis. DNA sequences were aligned manually and a haplotype network was created manually. Nucleotide diversity (π) was calculated using Arlequin v3.5.1.2 (Excoffier et al. 2005). AFLP loci were scored for presence/absence by the fragment analyzer software GeneMapper v 4.0 (Applied Biosystems) with a minimum of 50 relative fluorescent units (rfu), then manually screened, making this a semi-automatic scoring process as suggested by Papa et al. (2005). NTSYS-pc ver. 2.1 software (Rohlf 1994) was used to calculate the Dice (1945) similarity coefficient. Because some populations and regions contained identical AFLP genotypes, possibly because of clonal reproduction, we used the software GenoType/GenoDive (Meirmans and Van Tienderen 2004) to calculate the number of unique AFLP genotypes. Principal Coordinates Analysis (PCoA) was performed on Dice similarity coefficients using the DCENTER and EIGEN modules of NTSYS. Analysis of molecular variance (AMOVA), as implemented in Arlequin v3.5.1.2 (Excoffier et al. 2005), was used to examine the distribution of genetic variation of AFLP genotypes among and within North American and Eurasian populations from which five or

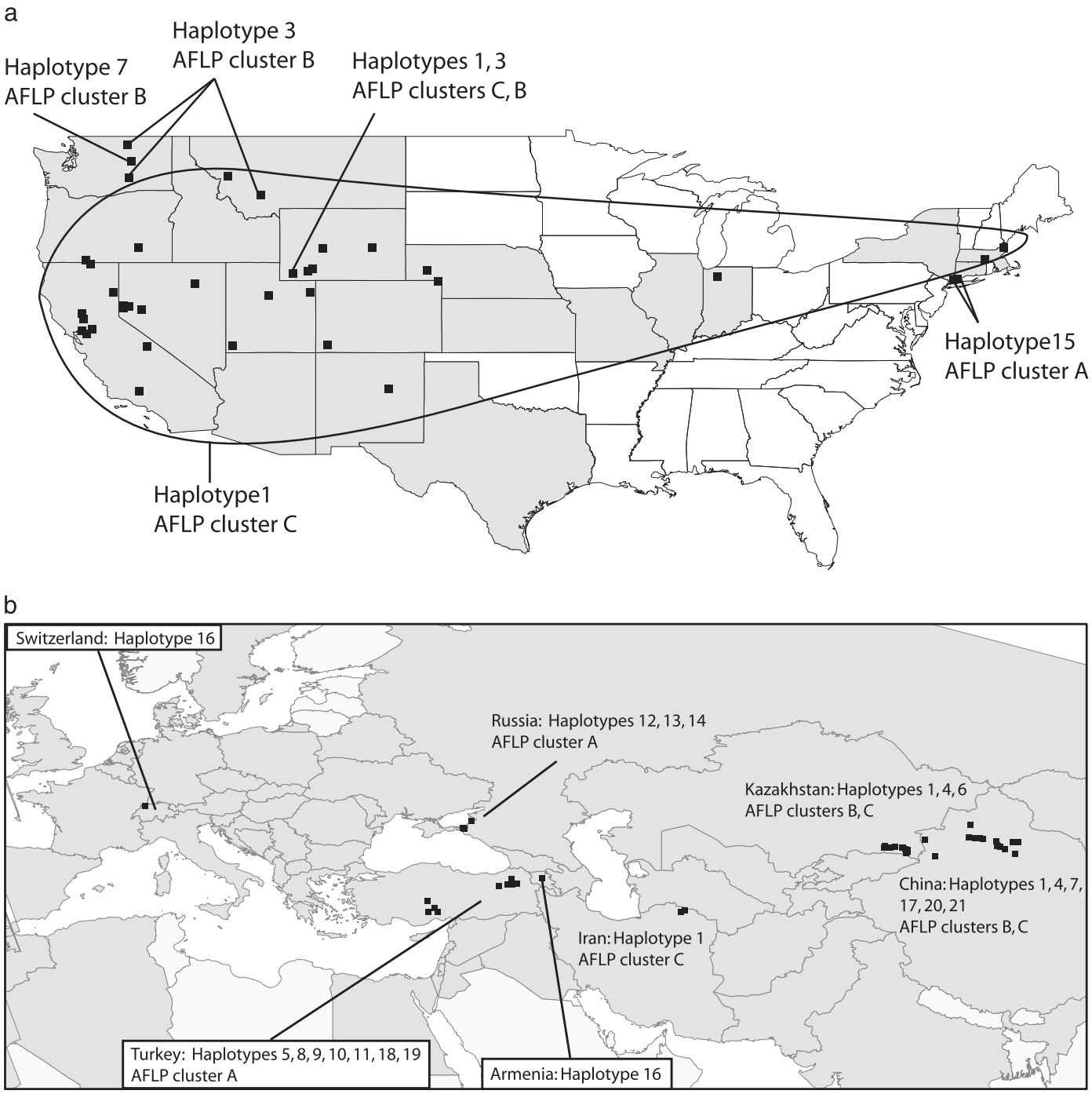

Figure 1. a) Map of populations of perennial pepperweed sampled in the United States. Shaded states indicate presence (USDA 2005), b) Map of *Lepidium latifolium* sensu lato collection locations in Eurasia. Darker and lighter shades of gray indicate presence or absence of *L. latifolium* in a country, respectively, according to GISD (2005). cpDNA haplotypes (see Figure 2) as well as AFLP cluster assignments (see Figure 4a) of any plants analyzed in a state or country are noted.

more plants were included in the analyses. Simpson's diversity index (D) corrected for sample size (Pielou 1969) was calculated for cpDNA sequences and AFLPs with

$$D = 1 - \sum n_i(n_i - 1)/N(N-1) \qquad \text{for } i = 1 \text{ to } G$$

where n_i is the number of plants that share genotype i. Values of D can range from 0 to 1, with higher values corresponding to greater genetic diversity. We used the Bayesian clustering and assignment software STRUCTURE v 2.3.3 (Falush et al. 2003, 2007; Pritchard et al.

Table 2. Genetic and nucleotide diversity for perennial pepperweed in the native and invasive ranges.

	cpDNA haplotypes				
	N[a]	G[b]	G/N	D[c]	Π[d]
Native range	139	17	0.12	0.12	0.03
Invasive range	91	5	0.05	0.04	0.02
	AFLP genotypes				
Native range	143	94	0.66	0.997	
Invasive range	288	8	0.03	0.427	

[a] N = number of plants.

[b] G = number of genotypes.

[c] D = Simpson's Diversity Index.

[d] π = Nucleotide diversity.

2000) to determine the number of clusters (K) of AFLP genotypes, and to determine how individual plants are assigned to these clusters. To determine K, we included 431 plants from the native and invaded region. For the analysis, specimens were diploidized (Falush et al. 2007), no population information was included, admixture was assumed, allele frequencies were considered to be correlated, and a 10,000 run burn-in (α stabilized by 1000 runs) and 10,000 run length were used. We tested for number of clusters (K) from 1 to 12 with 10 repetitions for each K. Selection of K from this output data was done by two methods: (1) using suggestions in the software documentation based on the mean estimated ln probability of the data, and (2) with a more formal criterion ΔK suggested by Evanno et al. (2005), and both of these methods were implemented in the software STRUCTURE HARVESTER webv0.6.92 (Earl and vanHoldt 2011). To determine how individual plants were assigned to these clusters, we used the same parameters in STRUCTURE as above, but used a 100,000 run burn-in and a 100,000 run length for the value of K determined above.

For 59 herbarium specimens representing populations from North America and Kazakhstan/China (where all three putative taxa are supposed to occur) we recorded morphological data for characters that have been used to separate the taxa in the native range (cauline leaf tip angle, pedicel pubescence, sepal persistence, shape of fruit base, ultimate fruiting branch structure, cauline leaf attachment and fruit surface pubescence (Table 1). Herbarium voucher specimens are deposited at Missouri Botanical Garden herbarium (MO).

Results

cpDNA sequence variation. The cpDNA region provided 655 bases of sequence data, 21 haplotypes (Table 2), and 52 variable sites including 45 single nucleotide polymorphisms and seven insertion/deletion (indel) events ranging from one to 14 base pairs (bp) in length. Five of the variable sites were homoplasious (found more than once on the haplotype network), and three of these were indels (1bp, 1 bp, 6 bp). Figures 1a and 1b indicate locations of cpDNA haplotypes. The 21 haplotypes created two main branches on the haplotype network, an upper and a lower one in Figure 2, separated by a string of 11 mutations including an 8 bp and a 14 bp indel. Most (64 out of 91) North American plants were haplotype 1, but we also found plants from Mexico (population 9242; see Appendix 3) that were haplotype 2, and plants from Connecticut populations (9083, 9084) that were haplotype 15, with all of these in the upper portion of the haplotype network. Plants from all populations in Washington (9050, 9053, 9056) and one population in Montana (12312) were haplotypes 3 and 7, found in the lower portion of the haplotype network. All populations from the invaded range were monotypic except population 9132 from Wyoming that contained haplotypes 1 and 3.

AFLP variation. The two AFLP primer pairs *Mse*I + CAT/*Eco*RI + AAG and *Mse*I + CTA/*Eco*RI + AAG, after manually screening, provided us with a total of 100 polymorphic fragments (52 and 48, respectively, see Appendix 4). There were 94 AFLP genotypes in 143 Eurasian plants, but only eight in 288 North American plants (Table 2). The eight genotypes from the invaded range varied in their Dice similarities from 0.99 to 0.13. Each North American genotype, except for one, was found in multiple plants, suggesting the variation between similar genotypes was most likely not caused by AFLP scoring errors. All North American plants with the same AFLP genotype had the same cpDNA sequence haplotype. Percentages of variation among and within populations were significantly different (AMOVA: $P < 0.0001$), with 97% variation among populations and 3% within populations. The amount of within population variation would have been 0% if not for the two mixed AFLP genotype populations in Wyoming and Montana (9132 and 12312; see AFLP data in Appendix 4). The percentage of genetic variation in Eurasia was 80% among populations and 20% within populations ($P < 0.0001$), and as in North America, all plants with the same AFLP genotype had the same cpDNA haplotype.

The results of STRUCTURE HARVESTER, using the mean estimated ln probability of the data, indicated that K = 3 (i.e. there are 3 clusters of AFLP genotypes). The ΔK method indicated that K = 2, 3, and 6 were clustering possibilities (Figure 3). At K = 2, 28 plants assigned as admixed between the two clusters (< 90% assignment to any cluster, data not shown), and at K = 3 (as shown in Figure 4a), only three plants assigned at < 90%. K = 6 (as shown in Figure 4b) describes further substructure of the

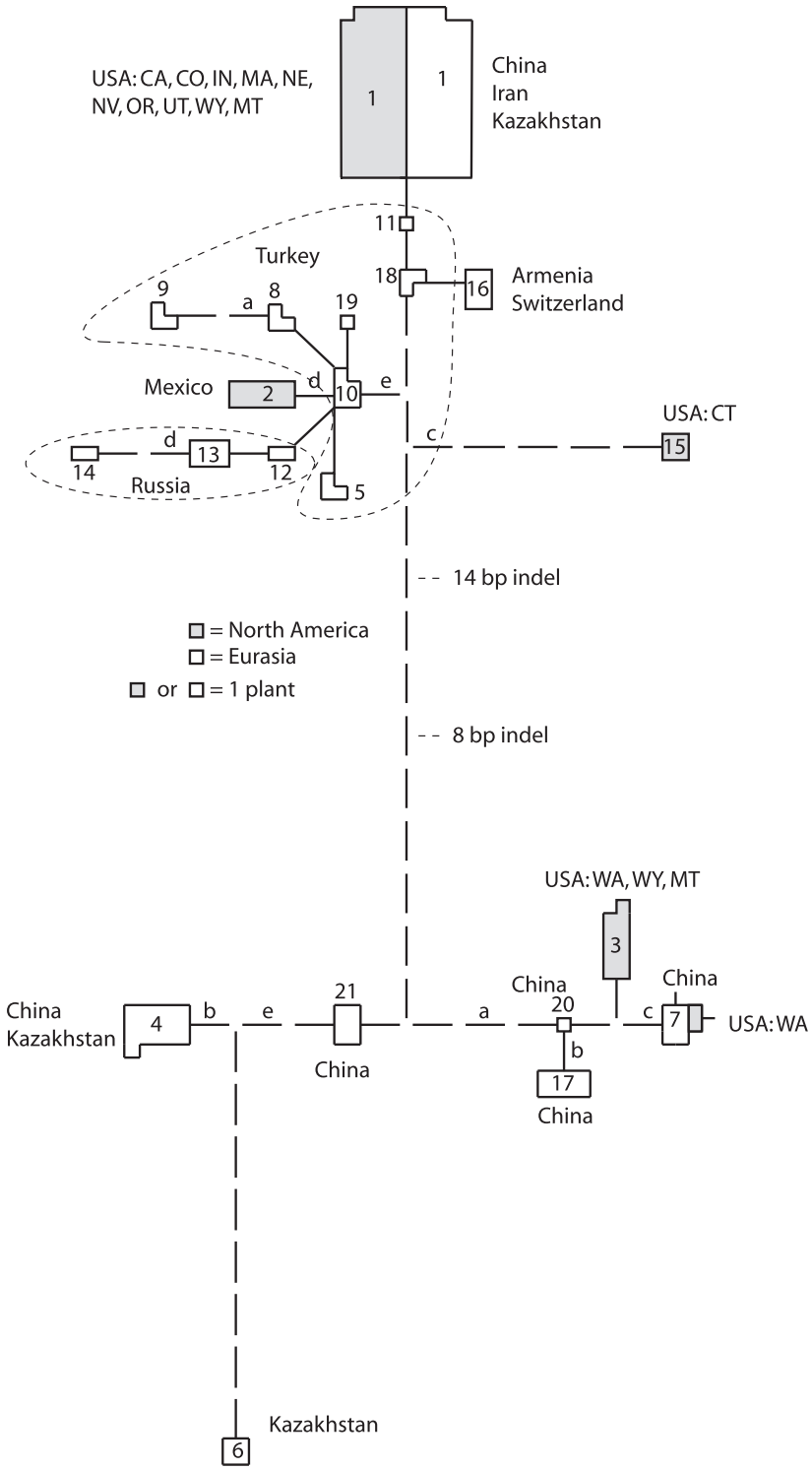

Figure 2. Haplotype network of cpDNA sequences from the *trnG* (UCC) - *trnS* (GCU) region for 230 *Lepidium latifolium* sensu lato plants. Haplotypes are represented as polygons, and these are separated by lines, each representing a mutation between the sequences. Lower case letters (a–e) indicate homoplasious mutations. Haplotype identification numbers are shown on or next to polygons, and size of the haplotype polygon is directly related to how many times that haplotype was recovered. The smallest polygon (e.g. haplotype 11) represents one plant, and the largest polygon (haplotype 1 from North America) represents 64 plants, and these two haplotypes are separated by one mutation. North American haplotypes are represented by shaded boxes, Eurasian haplotypes by white boxes. The two largest insertion/deletion (indel) events (8 and 14 bp) are noted. Dashed line circles indicate geographic origins of some haplotypes.

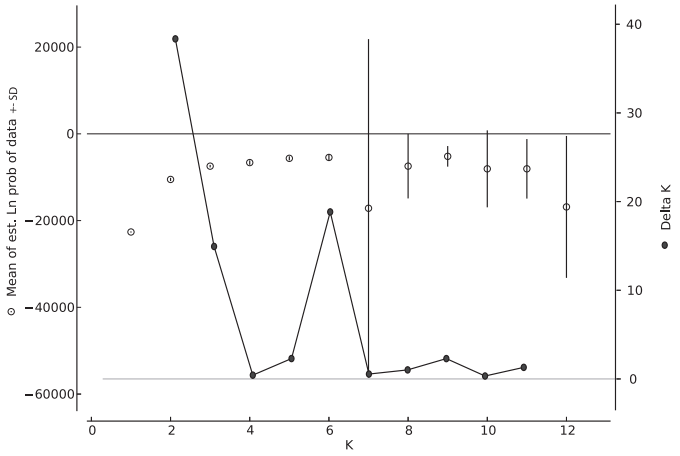

Figure 3. Graphs of mean posterior probability ± standard deviation of AFLP data for 10 runs of K = 1 to 12 (number of clusters) as determined by Bayesian clustering software STRUCTURE v 2.3.3 (Pritchard et al. 2000, Falush et al. 2003, 2007) for 431 perennial pepperweed plants from North America and Eurasia (open circles) and ΔK values for those same runs as determined by method of Evanno et al. (2005) (closed circle and line). The graph was created with the software STRUCTURE HARVESTER webv0.6.92 (Earl and vanHoldt 2011).

K = 3 clusters, with five plants assigned at < 90%. The substructure separates cluster B into three parts (b, c, d), and cluster C into two parts (e, f; Figure 4b).

Plants from North America assigned at > 90% to one of the three clusters. All 19 plants from two populations in Connecticut (9083, 9084; cluster A on Figure 4a) had identical AFLP genotypes, as did 226 U.S. plants ranging from California to Massachusetts (cluster C on Figure 4a). In cluster B, three Washington populations (9050, 9053, 9056) were monotypic, each with a unique AFLP genotype (Figure 4a). One Wyoming population (9132) had two genotypes in the B cluster, with the rest of the populations being identical to the U.S. plants in the C cluster. A population from Montana (12312) had two AFLP genotypes, both in the B cluster. When using K = 6 (further sub-structuring of K = 3 clusters, Figure 4b), one plant from North America failed to assign to a cluster at > 90% (9132.01 from Wyoming; 31% assignment to cluster b, 62% to cluster d), suggesting that the plant is the result of gene flow between plants in clusters b and d.

Morphology. Based on the species descriptions of *L. latifolium* and *L. obtusum* in Flora of China, and the character states associated with *L. affine* (which is discussed, but not recognized, in the Flora of China; Cheo et al. 2001), many herbarium specimens of plants from Kazakhstan and China had combinations of character states that did not fit any one taxon (24/30; Table 3). For example, we found plants with character states of *L. obtusum*, i.e., with persistent sepals (n = 10/30 plants) and cordate fruits (n = 2/30), but never together, and never with all of the other character states required to be identified as *L. obtusum* (see Table 1). Similarly, we found few plants that could conclusively be distinguished as *L. latifolium* or *L. affine* (n = 6/30 populations). In North America, where all plants are assumed to be *L. latifolium*, we also found variable combinations of character states, but few plants identified as *L. latifolium* sensu Flora of China (n = 4/24), and only one plant could be identified as *L. affine* (none identified as *L. obtusum*). All invasive samples fell within the recent *L. latifolium* species descriptions used in North America (Al-Shehbaz 2012; Al-Shehbaz and Gaskin 2010; Francis and Warwick 2007), none of which attempts to distinguish between *L. latifolium*, *L. obtusum* and *L. affine*.

The character states that could putatively separate the three taxa in the Kazakhstan and China collections did not correlate well with the cpDNA haplotypes or AFLP clusters found in our analyses. For example, persistent sepals (a character state of *L. obtusum*) were found in cpDNA haplotypes 1, 6, 7, 20 and 21 (Table 3, Appendix 5), and these haplotypes are spread across the haplotype network (Figure 2). Persistent sepals were also found in all AFLP clusters containing Kazakhstan/China genotypes (clusters B and C, Figure 4a). The same lack of correlation between morphology and genotype also applies to the other characters used to distinguish the species based on the identification key.

Discussion

Taxonomic confusion is not uncommon in invasive plants. Species such as leafy spurge have recently been listed under multiple scientific names (Crompton et al. 1990) that required clarification, and there are still cases where species names are unclear (e.g. Dalmatian toadflax; *Linaria dalmatica* (L.) Mill. or *L. genistifolia* (L.) Mill.; USDA 2005). We propose that we have helped clarify the identity of perennial pepperweed, as we found no support for distinguishing three species in the "*L. latifolium* sensu lato" group, i.e. no correlations between morphological characters used to distinguish these taxa and genotypic lineages or clusters were found. This indicates that either gene flow between the putative taxa is not restricted, or that evolution of our genetic markers does not correlate with evolution of the morphological characters. Also, the combinations of character states found in a plant, such as persistence of sepals, fruit shape, fruit and pedicel pubescence, etc., were often incompatible with the species descriptions in the Flora of China. We therefore agree with Cheo et al. (2001; Flora of China) that *L. affine* is likely an artificial taxon, and suggest that *L. obtusum* may also be artificial, pending further investigation of more extensive collections. Chro-

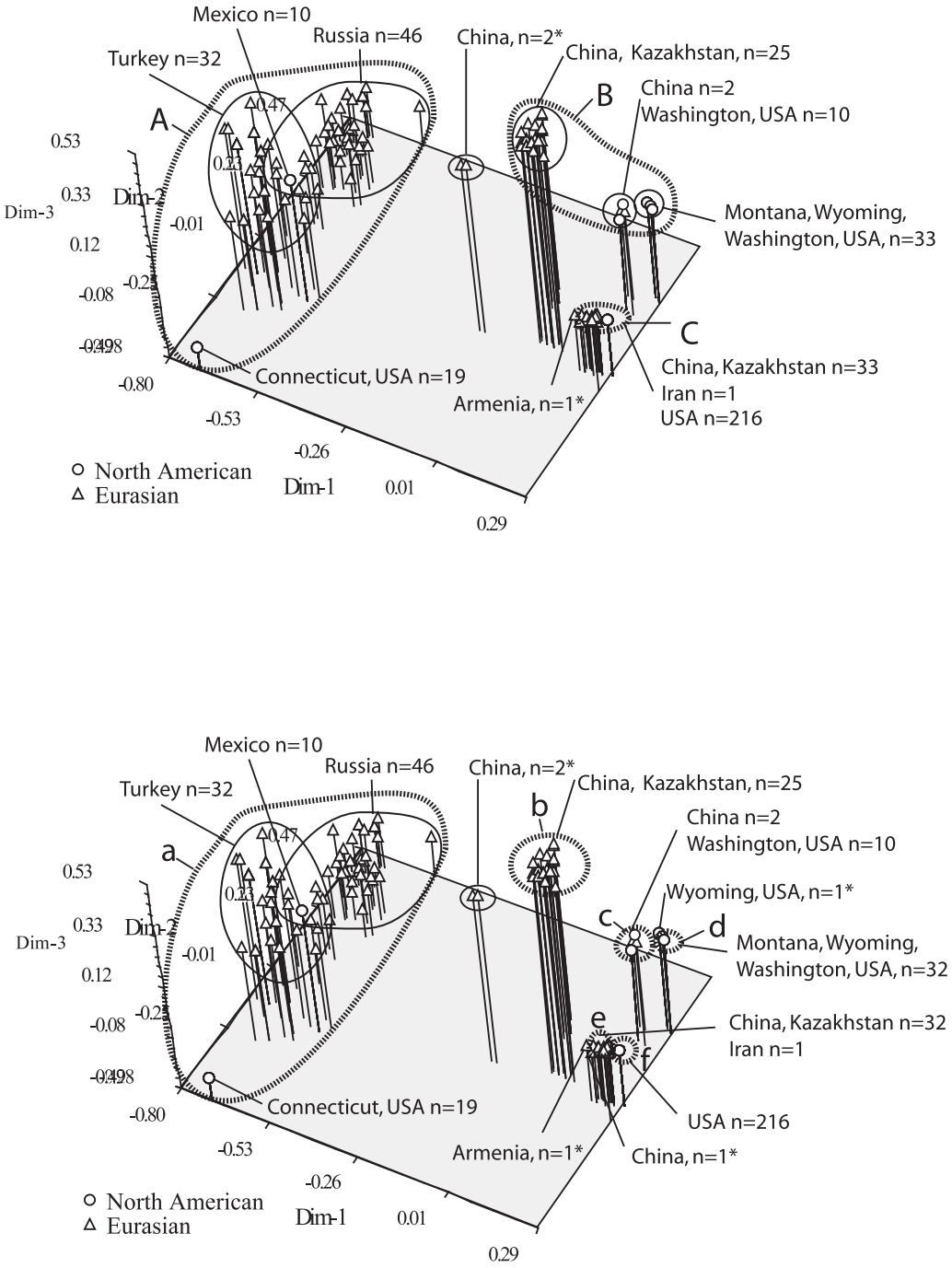

Figure 4. Principal Components Analysis (PCoA) of AFLP genotypes from 431perennial pepperweed plants from North America (circles) and Eurasia (triangles). In 4a, the number of identified clusters (K) is three (A–C). In 4b, the number of identified clusters is six (a–f). * indicates that a plant did not assign to any cluster at > 90%.

mosome counts should be included in further analyses as they putatively separate *L. latifolium* from *L. obtusum*, though there would still be a conflict between species descriptions and combinations of morphological character states we found in native plants.

As in the native range, the characters used in the Flora of China (Table 1) were not consistently useful for distinguishing species in the invaded range (Table 1), nor did they identify all plants as *L. latifolium*. However, all plants fell within the broader species description of *L. latifolium* used in the Flora of North America (Al-Shehbaz and Gaskin, 2010) which, like other North American treatments, does not acknowledge the presence of *L. affine* or *L. obtusum* or any sub-specific taxa within *L. latifolium*.

Table 3. Morphological characteristics of perennial pepperweed collections. For corresponding cpDNA haplotype and AFLP cluster assignment, see Appendix 5.

Population no.	Country	Cauline leaf tip	Pedicels	Sepals	Fruit base	Ultimate fruiting branches	Upper cauline leaves	Fruit surface	Species ID according to key in manuscript
4072	Armenia	acute	glabrous	deciduous	round	subcapitate	sessile	pubescent	?
10148	China	acute	glabrous	deciduous	round	subcapitate	sessile	glabrous	*L. affine*
10149	China	acute	glabrous	deciduous	round	subcapitate	sessile	glabrous	*L. affine*
10144	China	acute	glabrous	deciduous	round	subcapitate	sessile	pubescent	*L. latifolium*
10152	China	acute	glabrous	deciduous	round	subcapitate	subsessile	pubescent	*L. latifolium*
10141	China	acute	pubescent	persistent	round	subcapitate	sessile	glabrous	?
10145	China	acute	pubescent	deciduous	round	subcapitate	sessile	glabrous	?
10146	China	acute	pubescent	persistent	cordate	subcapitate	sessile	glabrous	?
10147	China	acute	pubescent	persistent	round	subcapitate	sessile	glabrous	?
10153	China	acute	pubescent	deciduous	round	subcapitate	sessile	glabrous	?
10154	China	acute	pubescent	deciduous	round	capitate	subsessile	glabrous	?
10155	China	acute	pubescent	persistent	round	capitate	sessile	glabrous	?
10156	China	acute	pubescent	immature	immature	subcapitate	sessile	immature	?
10142	China	acute	pubescent	persistent	round	subcapitate	sessile	pubescent	?
10143	China	obtuse	pubescent	deciduous	round	subcapitate	sessile	pubescent	?
10150	China	acute	pubescent	persistent	round	subcapitate	subsessile	pubescent	?
10151	China	acute	glabrous	deciduous	round	subcapitate	sessile	pubescent	?
10157	China	acute	pubescent	deciduous	round	subcapitate	subsessile	pubescent	?
10123	Kazakhstan	acute	glabrous	deciduous	round	subcapitate	sessile	glabrous	*L. affine*
10128	Kazakhstan	acute	glabrous	deciduous	round	subcapitate	sessile	glabrous	*L. affine*
10127	Kazakhstan	obtuse	glabrous	persistent	round	subcapitate	sessile	glabrous	?
10130	Kazakhstan	acute	glabrous	deciduous	cordate	subcapitate	sessile	glabrous	?
10133	Kazakhstan	acute	glabrous	deciduous	round	subcapitate	sessile	glabrous	?
10138	Kazakhstan	acute	glabrous	deciduous	round	subcapitate	subsessile	glabrous	?
10139	Kazakhstan	acute	glabrous	deciduous	round	subcapitate	subsessile	glabrous	?
10121	Kazakhstan	acute	pubescent	deciduous	round	subcapitate	sessile	pubescent	?
10125	Kazakhstan	acute	glabrous	persistent	round	capitate	sessile	pubescent	?
10126	Kazakhstan	acute	glabrous	persistent	round	capitate	sessile	pubescent	?
10132	Kazakhstan	acute	glabrous	deciduous	round	subcapitate	sessile	pubescent	?
10134	Kazakhstan	acute	glabrous	persistent	round	subcapitate	sessile	pubescent	?
10137	Kazakhstan	acute	pubescent	deciduous	round	subcapitate	subsessile	pubescent	?
8773	Turkey	acute	glabrous	immature	immature	subcapitate	subsessile	immature	?
8774	Turkey	acute	glabrous	immature	immature	subcapitate	subsessile	immature	?
8775	Turkey	acute	glabrous	immature	immature	subcapitate	subsessile	immature	?
8776	Turkey	acute	glabrous	immature	immature	subcapitate	subsessile	immature	?
9176	USA	acute	glabrous	deciduous	round	subcapitate	sessile	glabrous	*L. affine*
9062	USA	acute	glabrous	deciduous	round	subcapitate	subsessile	pubescent	*L. latifolium*
9083	USA	acute	glabrous	deciduous	round	subcapitate	subsessile	pubescent	*L. latifolium*
9084	USA	acute	glabrous	deciduous	round	subcapitate	subsessile	pubescent	*L. latifolium*
9151	USA	acute	glabrous	deciduous	round	subcapitate	subsessile	pubescent	*L. latifolium*
9056	USA	acute	glabrous	both	round	subcapitate	sessile	glabrous	?
9060	USA	acute	glabrous	deciduous	round	subcapitate	subsessile	glabrous	?
9132	USA	acute	glabrous	both	round	subcapitate	sessile	glabrous	?
9136	USA	acute	glabrous	deciduous	round	subcapitate	subsessile	glabrous	?
9165	USA	acute	pubescent	deciduous	round	subcapitate	subsessile	glabrous	?
9182	USA	acute	glabrous	both	round	subcapitate	sessile	glabrous	?
4307	USA	acute	pubescent	immature	immature	subcapitate	subsessile	immature	?
9050	USA	acute	glabrous	immature	immature	subcapitate	sessile	immature	?

Table 3. Continued.

Population no.	Country	Cauline leaf tip	Pedicels	Sepals	Fruit base	Ultimate fruiting branches	Upper cauline leaves	Fruit surface	Species ID according to key in manuscript
9057	USA	acute	glabrous	immature	immature	subcapitate	subsessile	immature	?
10075	USA	acute	pubescent	immature	immature	subcapitate	sessile	immature	?
4628	USA	acute	pubescent	deciduous	round	subcapitate	subsessile	pubescent	?
9053	USA	acute	pubescent	persist	round	subcapitate	sessile	pubescent	?
9081	USA	acute	pubescent	deciduous	round	subcapitate	sessile	pubescent	?
9082	USA	acute	pubescent	deciduous	round	subcapitate	subsessile	pubescent	?
9130	USA	acute	pubescent	deciduous	round	subcapitate	subsessile	pubescent	?
9143	USA	acute	pubescent	deciduous	round	subcapitate	subsessile	pubescent	?
9149	USA	acute	pubescent	deciduous	round	subcapitate	sessile	pubescent	?
9167	USA	acute	pubescent	deciduous	round	subcapitate	subsessile	pubescent	?
9178	USA	acute	pubescent	deciduous	round	subcapitate	sessile	pubescent	?

Knowing population structure and diversity of weeds can also improve invasive weed management, by revealing whether multiple or single introductions have occurred (e.g. Marrs et al. 2008; Xu et al. 2010), and helping describe origins of the invasion, which is critical for finding locally-adapted biological control agents (e.g. Evans and Ellison 2004; Goolsby et al. 2006). In an earlier analysis of mode of reproduction in this species, Gaskin et al. (2012) noted that plants analyzed from a limited collection in the native range, i.e. Turkey and south-western Russia, were not genetically similar to plants invading the United States. In this project, the inclusion of plants from some other parts of the native range serendipitously found that the most common North American genotype (in AFLP cluster C, n = 216) was very similar (Dice similarity of 0.98) to plants in one Kazakhstan (10127) and two China populations (10150, 10151). In addition, the five genotypes in AFLP cluster B (Montana, Washington and Wyoming) were similar to plants in a population from China (10146; Dice similarities of 0.88 to 0.99). In contrast, genotypes from Mexico and Connecticut had much lower similarities to plants we sampled in Eurasia, with the Mexico genotype best matching a genotype from Turkey (Dice = 0.64, 9802), and Connecticut best matching a genotype from Russia (Dice = 0.58, 10525). This result also indicates that our collections in the native range are incomplete, and better or more widespread origins of the invasion may be found through more extensive sampling. Nevertheless, the close similarities between some genotypes in the United States and Kazakhstan and China suggest that phytophagous organisms best adapted to the most common invasive genotypes might be found there. So far, candidate agents under investigation originate from Turkey and southern Russia (Hinz et al. 2008) and preliminary results suggest that all U.S. perennial pepperweed cpDNA haplotypes are accepted as hosts by three potential agents (Gerber and Hinz, unpublished data). If additional agent searches are eventually required, that work would be focused on Kazakhstan and China.

The center of diversity of *Lepidium latifolium* s.l. is not clear. A relatively high number of cpDNA haplotypes were found in Turkey and southwestern Russia, and mostly different haplotypes were found in China and Kazakhstan. Haplotypes from those regions are mostly from different parts of the haplotype network (top and bottom respectively) suggesting a wide native range. The one exception is the common haplotype 1 which is found in the top of the haplotype network but is common in China and Kazakhstan, and was not found by us in Turkey or Russia. This suggests the possibility that haplotype 1 may have been introduced to China and Kazakhstan from further west, which could imply that there is a different historical origin of that haplotype, and that origin may contain a more diverse assemblage of natural enemies of haplotype 1 than are found in the eastern portion of the species range.

Within *L. latifolium* in North America, we were able to describe five cpDNA sequence haplotypes in 91 plants, and eight AFLP genotypes in 288 plants. This low number of haplotypes and genotypes compared to the native range is most likely because of a limited number of introductions or a severe bottleneck, and subsequent vegetative spread, or sexual reproduction between highly similar or identical genotypes (Gaskin et al. 2012, Renz et al. 2012). Plants with identical AFLP genotypes always had identical cpDNA haplotypes, even in the mixed genotype population (9132), suggesting that few or no new genotypes are being created in North America. In a recent study Gaskin et al. (2012) found that plants from six populations in the United States (all haplotype 1, AFLP cluster C) were unable to create seed via apomixis but were very successful at producing seed by selfing and outcrossing. As they were

all genetically identical to each other for the markers used in the study, even outcrossing could result in F1 individuals identical to both parents, which may help explain the low number of genotypes found in the United States. There have been numerous studies comparing genetic diversity in the native and invasive range of species and results varied from an increase in diversity (e.g. brown anole: Kolbe et al. 2004), to a retention of diversity similar to the native range (e.g. common ragweed: Genton et al. 2005; hoary cress: Gaskin et al. 2005), to only one genotype present in the invasion (e.g. Japanese knotweed: Hollingsworth and Bailey 2000; alligator weed: Li and Ye 2006). This variation in genetic diversity within invasive species suggests that invasion processes among successful invaders can vary greatly (Roman and Darling 2007).

The low diversity of this invasion may help management efforts, because a limited number of genotypes in North America suggests limited opportunities for evolution of resistance or tolerance to an effective chemical or biological control method. Also, as mentioned above, we identified and supplied seed representing the cpDNA haplotypes found in the United States for use in host-specificity and efficacy studies of potential biological control agents. Including all or a good representation of the genetic diversity of an invasion in host-specificity testing should reduce the risk of any resistance to biological control agents being found after release.

In conclusion, our results indicate that *L. latifolium* most probably consists of one taxonomic unit in its native and invaded ranges. The majority of genetic variation in North America was found among populations and there were only eight cpDNA genotypes, suggesting few introductions or a severe bottleneck, and little or no creation of new genotypes since introduction. Origins of most invasive genotypes are likely in Kazakhstan and China, suggesting that these regions should preferentially be searched in future foreign exploration for additional biological control agents.

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